Ultrasound Structure and Digital Image Analysis of the Subepidermal Low Echogenic Band in Aged Human Skin: Diurnal Changes and Interindividual Variability

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Subepidermal low-echogenic band is a consistent echostructural finding in aged and photodamaged skin. The thickness of subepidermal low-echogenic band is considered to closely reflect the degree of cutaneous aging and its use for the monitoring of the severity of photoaging and the efficacy of drugs is rapidly expanding. We investigated subepidermal low-echogenic band structure in 23 old healthy volunteers (ages 75–100) with a high-frequency ultrasound scanner (B-mode, 20 MHz). Images were collected from the volar forearm twice daily: in the morning before getting up and 12 h later. To assess the severity of subepidermal low-echogenic band, echogenicity of the subepidermal region was determined by the image analysis and compared with visual scoring and subepidermal low-echogenic band thickness. All three methods gave consistent results, image analysis being the most sensitive, reliable, and bias-free. Significant interindividual variability of subepidermal low-echogenic band echostructure was revealed. Moreover, circadian variability of subepidermal low-echogenic band echogenicity was observed. These major interindividual and diurnal variations of subepidermal low-echogenic band suggest that this band does not only represent an irreversible structural change but also a redistribution of fluid in the aged dermis. Diurnal variations in the subepidermal low-echogenic band would limit the use of this parameter in studies of skin aging, photoaging, and efficiency of medication. *Key words: high-frequency ultrasonography. J Invest Dermatol 102:362–365, 1994*

A precise non-invasive quantification of aging and photodamage of human skin would be extremely useful but has proved difficult to achieve. Recently introduced high-frequency ultrasonography seems to be a promising method for this purpose. In the ultrasound image of the aged skin a subepidermal low-echogenic band (SLEB) is a frequent finding [1]. It has been reported that its thickness increases proportionally to the age [2] and it was proposed to use SLEB thickness as a convenient parameter to measure skin aging and photoaging and to evaluate the efficacy of medications [2–5]. Little is known about the origin of SLEB and its structural basis. Elastosis or changes in collagen structure were suggested to be partially responsible for an echolucent material formation [1,2]. Moreover, in view of the recent magnetic resonance studies it is likely that subepidermal region of the aged skin is especially prone to accumulate increased amounts of water ([6], S. Richard, to be published).

To critically assess the usefulness of SLEB for the quantification of skin aging we describe here its prevalence and structure in a defined population of old volunteers. Different methods of SLEB assessment were employed and compared: visual scoring, thickness measurements, and computer-assisted image analysis. The latter enables objective measuring of the echogenicity of the subepidermal skin region containing SLEB. We found that the computer-assisted image analysis of SLEB described well its echostructure in a way that was free from subjective bias of visual scoring and thickness measurements. The results showed that SLEB presented considerable interindividual structural variability. We also showed here that SLEB thickness and echostructure varied considerably during the day. Moreover, we discuss the significance of the diurnal variations of SLEB echostructure to the understanding of its origin.

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Abbreviations: LEB, low-echogenic pixel; SLEB, subepidermal low-echogenic band.

MATERIALS AND METHODS

Subjects 23 old volunteers (ages 75–100, median 89; 17 women, 6 men) were recruited in a nursing home. They all gave their informed consent to take part in the study. Ethical approval for the study was given by the Ethic Committee of Copenhagen. The investigations were performed in the winter season and at least 1 month before the examination forearm skin was not exposed to sun or ultraviolet light.

Ultrasonographic Recordings A 20-MHz ultrasonograph (Dermascan C, Cortex Technology, Denmark) [7] was used to obtain cross sectional images of the skin (B mode). The instrument consists of the three main parts: the C probe with the transducer mounted on an adjustable balanced supporting arm, the elaboration and visualization system, and the data-storing system. The probe is placed on the skin in a fixed standard position. The ultrasonic wave is partially reflected at the boundary between adjacent structures, and generates echoes of a defined amplitude. The intensity of the reflection echoes is evaluated by the microprocessor and visualized on a colored two-dimensional image. The color scale of echogenicity is white > yellow > red > green > blue > black. The gain curve was adjusted in the horizontal position at 22 dB. This gain had been previously found to give the maximal A-scan peaks on the rubber phantom provided by the manufacturer. For the four possible zoom levels the first horizontal magnification

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was chosen. The velocity of ultrasound in the skin was 1580 m/second. We investigated the region of the middle volar forearm. For the time of measurement the examined place was marked with an adhesive ring (Beiersdorf) that allowed to obtain a uniform image size. To ensure exactly the same position of the ring during the second measurement, the place was additionally marked with a dermograph. The probe was placed on the skin in a fixed standard position. The axis of the transducer was perpendicular to the skin surface, and this position was assured by checking the parallel orientation between the ultrasound images of membrane of the probe and the epidermal entrance echo. Images were taken in duplicate twice daily, in the morning before getting up and in the evening (12 h after the first measurement). Between each of the duplicate measurement forearm was removed from the "Dermascan" probe.

Visual Scoring Ultrasound skin images were photographed with a Polaroid camera directly from the screen. To avoid bias these pictures were mixed with images of young skin and then scored in a random order and blinded manner by five individuals with a previous experience in skin ultrasonography, without the knowledge of the nature of the experiment. Single pictures were scored on a four-point scale (See Fig 1 for reference): 0, no SLEB visible; 1, low-echogenic (black) spots arranged linearly under epidermis; 2, low-echogenic subepidermal confluent patches; 3, clear low-echogenic band. Then, both pictures (morning and evening) of the same person were presented simultaneously to assess diurnal changes of SLEB echostructure. The following three-point scale was used: 1) diminution of SLEB, 2) enlargement/expansion of SLEB, 3) no change. The median scores were selected for subsequent analyses.

Measurement of SLEB and Whole Skin Thickness The region of SLEB was marked with a cursor by hand outlining of the subepidermal low-echogenic area. SLEB thickness was determined by dividing the area of SLEB by the length of the overlaying epidermis. Skin thickness was computed similarly as a quotient of skin area and the horizontal.

Computer-Assisted Image Analysis Echographic images were recorded on the floppy discs and further processed by a dedicated computer software (GIPS, Cortex Technology, Hadsund, Denmark). In this system the amplitudes of the echoes of the single image elements (pixels) are ascribed to a numerical scale (0–255). The low-echogenic area extends from 0–30 [8]. The number of low-echogenic pixels (LEP) was counted in the skin area extending 0.75 mm under skin surface.

Statistical Analysis All the confidence intervals represent 95%. The standard deviations of the methods of measurement were calculated from the data. The kappa statistics were used to measure the agreement between different systems of SLEB evaluation methods. To assess the correlation between SLEB thickness and pixel numbers Spearman correlation coefficient with confidence intervals was computed. The relation between individual age and sex on SLEB pixel number was evaluated with regression analysis. The regression and covariance analysis was used to evaluate the daily changes of SLEB pixel values.

RESULTS

Visual Scoring of the SLEB In the examined group of old volunteers a considerable variation in SLEB echostructure was present. SLEB was detected in all the individuals, but in four cases (17%) it was described as weak and faint (score 1) and in seven individuals (30%) the score was 2. Thus well-developed SLEB was observed only in 53% (confidence interval 42–64%) of cases. A separate scoring system was used to evaluate diurnal changes of SLEB morphology. Only in six cases (26%) was it judged that its echogenicity did not change. In the remaining cases SLEB echogenicity changed: in 11 individuals it was weaker and more narrow in the afternoon, whereas in 6 the opposite phenomenon was noted, a marked widening and expansion of the SLEB (Fig 1). Thus in 74% of individuals (confidence interval 56–92%) SLEB echostructure showed clear diurnal variation. Each visual score employed gave a high interrater accordance (0.83 and 0.88, respectively).

SLEB Thickness Measurements The precision of this measurement, defined as the standard deviation of duplicate thickness determinations in one site, was 0.07 mm. Similarly to the visual scoring results, measurements of the thickness of SLEB gave variable results. The measurements of grade 1 and 2 SLEB was often impossible because of ill-defined borders. The range of the measurements spanned 0 mm (SLEB thickness within the 2 SD of the method) to 0.71 mm (95% confidence interval 0.40–0.52). The afternoon measurements yielded a similar variability (range 0–0.67 mm, 95% confidence interval, 0.33–0.55 mm). The within patient changes of SLEB thickness, expressed as a difference between afternoon and morning value differed significantly in particular individuals. In eight volunteers the SLEB thickness increased for the value that exceeded 2 SD of the method of skin thickness measurements, whereas in nine it decreased. The agreement between SLEB changes scoring and changes in the thickness was good; kappa statistics equalled 0.6.

Computer-Assisted Image Analysis of the Subepidermal Region With the aid of computerized image analysis LEP number was counted in the subepidermal area. The precision of this method was 36 pixels. The range of morning and afternoon LEP values were 303–2299 (median 1167) and 255–2347 (median 1133), respectively. The agreement between scoring and pixel values was measured. For grade 1 the mean value of LEP number was 559 (confidence interval 243–723), for grade 2 it was 891 (confidence interval 746–1036), and for grade 3 1632 (confidence interval 1456–1807). The values of SLEB thickness also correlated well with the subepidermal echogenicity values (Fig 2); the Spearman rank correlation coefficient equalled 0.88, confidence interval 0.72–1.0 ($p = 0.002$). During the day meaningful changes of pixel values (exceeding 2 SD of the method) were detected in all individuals and these alterations resembled the diurnal changes of SLEB thickness. The agreement between the score for the change of SLEB thickness and the diurnal changes of pixel values was moderate (kappa 0.51). Pixel measurement was more sensitive than scoring in detection of changes of SLEB echogenicity.

Influence of Initial Skin Echogenicity on the Subsequent Echogenicity Changes in the Subepidermal Area From the analysis of the ultrasonographic images we noted a negative association between morning skin echogenicity and subsequent change in echogenicity, namely, individuals with low morning LEP count were often found to have high LEP values in the afternoon, the opposite being also true. This observation was therefore tested formally. The hypothesis about the association between the morning LEP value and the direction of echogenicity change during the day was examined by fitting a linear regression model [9] (Fig 3). We found that diurnal LEP change is negatively associated with the morning LEP value (regression line slope −0.43, SD 0.03, $p = 0.002$). The residuals showed normal distribution with the mean of 0. The correction for regression to the mean phenomenon was performed by computing the corrected value of the slope [10]. The corrected value of slope was still significant, −0.42.

Covariance Analysis The covariance analysis revealed that there was no relationship between the LEP number in the subepidermal area and age or sex of the individuals. Moreover, diurnal changes of pixel numbers were not dependent on age or diuretic use. A marginal relation between sex and diurnal changes of pixel values was noted; female subjects tended to have higher LEP values in the afternoon than in the morning ($p = 0.06$).

DISCUSSION

In this study we evaluated the echostructure of SLEB using digital image analysis and we compared this method with visual scoring and SLEB thickness. We report here for the first time prominent circadian changes of SLEB echostructure and describe a considerable interindividual variability of the ultrasound structure of this band.

SLEB has been used for the quantification of skin damage due to aging and/or a light exposure despite its unclear origin [2–5]. Furthermore, the thickness of SLEB was previously shown to be predictable from the age of the individual [2]. However, our preliminary investigations showed that SLEB was very variable in structure: it might present in the form of subepidermal echolucent spots, confluent patches, and finally a continuous band. In this study the SLEB heterogeneity was confirmed and quantified with the use of the visual scale. As much as 47% of the individuals in our group had SLEB with an interrupted structure and ill-defined borders. In

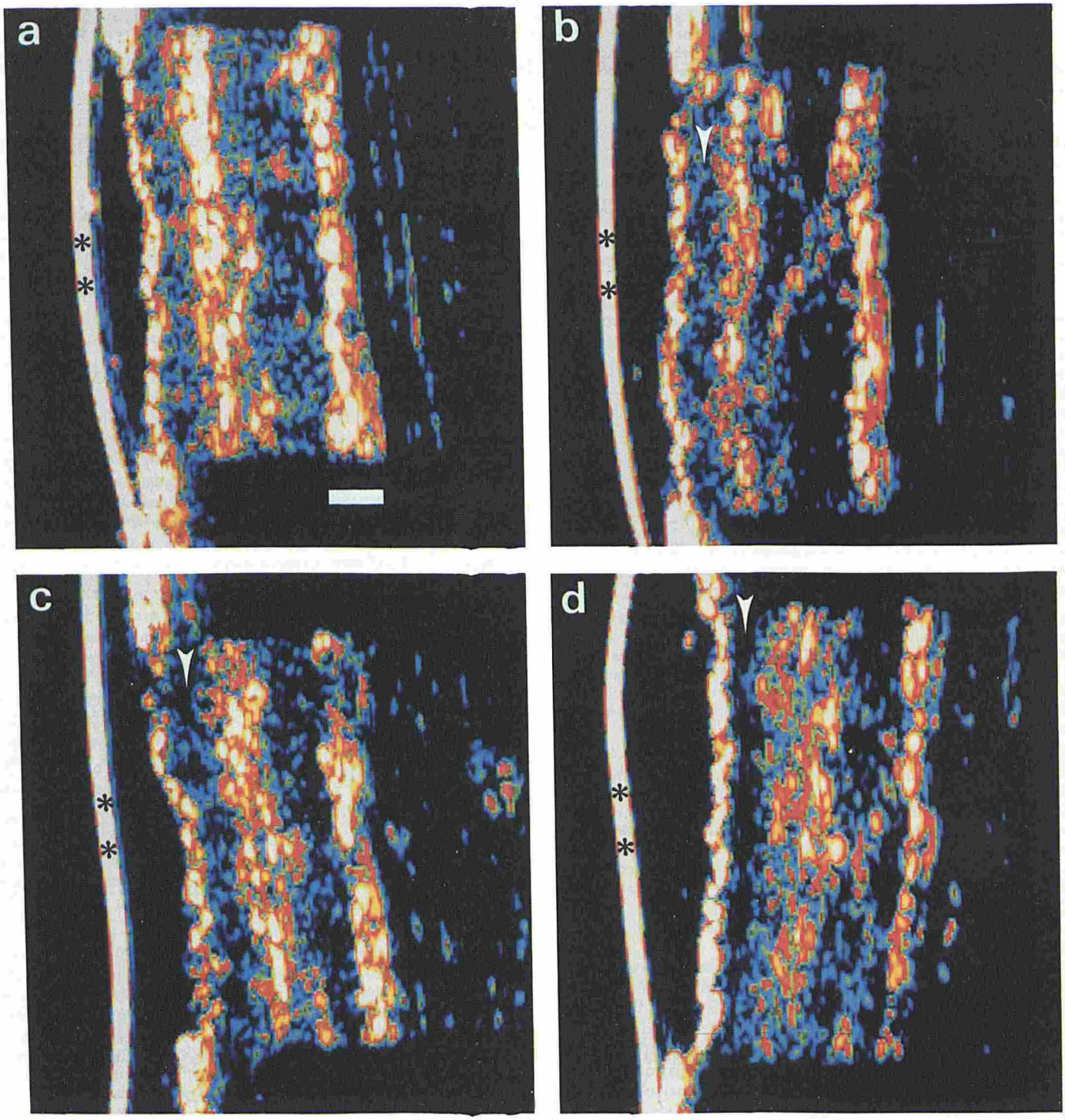

Figure 1. Ultrasonographic images of forearm skin. Subepidermal low-echogenic band is marked with *arrows*. Visual scoring of SLEB: *a*, grade 0 (no SLEB); *b*, grade 1 (subepidermal echolucent spots); *c*, grade 2 (subepidermal echolucent patches); *d*, grade 3 (continuous SLEB). Images *b*, *c*, and *d*, *a* were obtained from the same persons in the morning and afternoon, respectively. ** Membrane of the ultrasound probe. *Bar*, 0.5 mm.

these cases the measurement of SLEB thickness may be difficult and unreliable. Moreover, because of SLEB heterogeneity both visual scoring and thickness measurements are not free from a significant subjective bias. Probably due to these factors some investigators were unable to find a significant relation between SLEB thickness and age (Schatz H, Stoudemayer T, Gabriel K, *et al.* Visualization of aging and photodamage in human skin by 20 MHz ultrasound. 8th International Symposium on Bioengineering and the Skin, Stresa, Italy, June 13–16, 1990). To avoid a bias in SLEB assessment, a computer-assisted digital image analysis of the skin was used for the first time for quantification of SLEB severity. In this system the number of low-echogenic elementary picture elements (pixels) was computed from a predetermined area of ultrasonographic image (in our case the area extended 0.75 mm below the epidermal surface). Because SLEB is per definition echolucent it is conceivable that the quantitation of low-echogenic pixels (LEPs) may provide an accurate measure of its severity. We found that the number of LEPs was significantly correlated with the visual score and the SLEB thick-

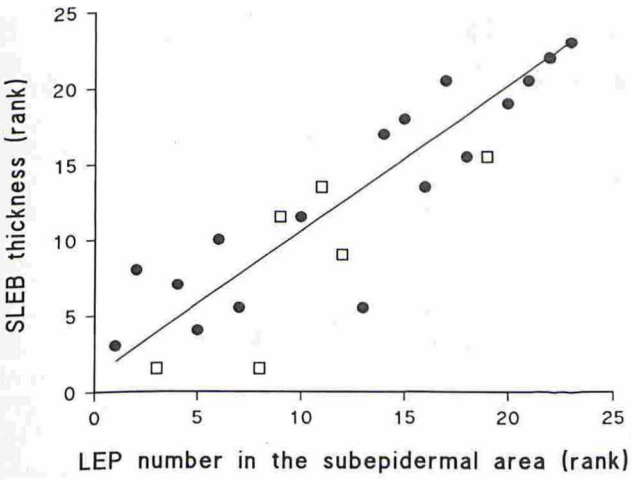

Figure 2. Correlation between thickness of SLEB and number of low-echogenic pixels (LEP) in the subepidermal area. The measurements of SLEB thickness and image analysis were performed as in *Materials and Methods. Open squares,* men; *closed circles,* women. The Spearman correlation coefficient = 0.88.

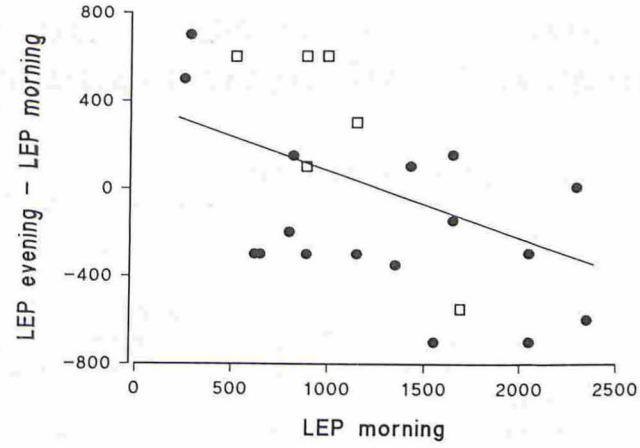

Figure 3. Diurnal changes of SLEB echostructure. SLEB images were obtained in the morning and evening and low-echogenic pixel (LEP) numbers counted as described in *Materials and Methods.* The diurnal change of echogenicity ($LEP_{evening} - LEP_{morning}$) are regressed against the baseline ($LEP_{morning}$) values. *Open squares,* men; *closed circles,* women. The slope (corrected for the regression for the mean phenomenon equals), −0.42 (significant at $p = 0.002$).

ness. Despite a uniform experimental group the visual scoring, thickness measurements, and image analysis revealed that the structure of SLEB differed significantly between individuals. The covariance analysis revealed that these differences were not related to the age and sex. Because for the investigations we have chosen the ventral side of the forearm that is not directly exposed to the sun, extensive differences in the photodamage were also an unlikely explanation of this heterogeneity.

This study revealed that SLEB structure underwent diurnal changes. It was found that the pattern of the diurnal change depended on the initial (morning) SLEB structure: individuals with high morning pixel counts tended to have low pixel numbers in the evening. Diurnal changes were not related to the age, sex, and diuretic drug use (covariance analysis). They were also unlikely to be caused by the lack of accuracy of the measurement, because the magnitude of the changes considerably exceeded the standard deviation of the method. Image analysis was more sensitive to detect variability in SLEB than visual scoring and thickness measurements. Therefore, image analysis of SLEB is in our opinion the method of choice in the quantitative and bias-free assessment of the SLEB.

The phenomenon of diurnal changes of SLEB may add to the explanation of its origin. It was previously established that echogenicity of the skin depends on the dermal water content, i.e., the extensive accumulation of water produces more echolucent structure. For instance in a place of a positive patch test the equivalent of SLEB develops as a result of inflammatory oedema in the papillary dermis [7,8]. Therefore it is conceivable that time-dependent echostructural changes of SLEB structure reflect alterations in dermal water distribution. The structural changes in the subepidermal region of senile skin may play a permissive role for these water shifts. Especially relevant is the observation of increased glycosaminoglycan content in the upper dermis of the photodamaged skin [11]. Glycosaminoglycans may bind large volumes of water, thus producing the effect of subepidermal echolucency. In hairless mice we have found recently that the accumulation of glycosaminoglycans in the papillary dermis is associated with increased skin water content and ultrasonographic picture of subepidermal low echogenic band (to be published). It is therefore likely that water bound by glycosaminoglycans contributes to the SLEB formation. Our explanation is supported by the recent finding of increased water content in the papillary dermis of aged skin by magnetic resonance [6]. The origin of the diurnal changes of SLEB structure cannot be explained at present: it is possible that the gravitational influences on body water balance or/and ill-defined local mechanisms may operate.

The diurnal changes of SLEB structure will probably limit the usefulness of SLEB evaluation for the assessment of skin aging and the action of pharmaceuticals on senile skin. A caution should be exercised in the interpretation of the studies where SLEB monitoring is a key endpoint parameter. The design of such studies should include measurements on the subjects at the same time of the day. Finally, it must be emphasized that the decrease of SLEB thickness or severity during treatment does not necessarily mean the structural repair of the senile skin but may just reflect altered water balance in the papillary dermis.

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